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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

LONGEVITY: TRANSLATION OF AGING THEORIES INTO ACTION

by

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DEDICATION

I would like to thank Kfar Saba, USA, because it truly takes a tribe to raise a child. My Ema (Mom) who is my role model and the epitome of creating your own success. She constantly pushed me to give my all, never quit, and stay focused on the ultimate goal. My stepfather Arbel who taught me that I should only work towards what is important to me, and to work hard. My father, who is the embodiment of remaining curious, preaches the importance of human connection, and is always there near or far. My older brother Ofek for taking my ideas seriously, feeding the ambition within me, and instilling confidence in my ability to impact the world. My girlfriend Ivana; my partner through it all; for supporting my goals which may often take me away from her, for showing me what it means to be independent, resilient, and strong, and for being such a motivational force to be around. This is the beginning of my journey. And my journey is for all of them.

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LONGEVITY: TRANSLATION OF AGING THEORIES INTO ACTION

ROY MILLER

ABSTRACT

Healthspan describes the length of time an individual lives without disability or chronic disease. Characteristic to aging is the risk for the onset of both through a progressive accumulation of deficits in normal physiologic function. In the past, the declines associated with aging were simply accepted as inevitable. Today, longevity research has undergone a meteoric rise in popularity. This is due to several landmark studies demonstrating that what was once thought of as inevitable has potential to be delayed. This thesis aims to consolidate current theories of biochemical processes believed to underlie aging, and explore their interconnections. Furthermore, current pharmaceutical and lifestyle interventions being studied to promote longevity and target these specific pathways will be analyzed for safety and practicality for use in a primary care setting. Through a shift from symptom-based care to personalized preventative care, the goal is to maximize function into older age and empower individuals to live life to the fullest well beyond what was previously imagined.

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LIST OF ABBREVIATIONS

AA.....	Amino Acid
AFAR.....	American Federation for Aging Research
AMPK.....	5' AMP- Activated Protein Kinase
CREB	cAMP Response Element-Binding Protein
CRTC2	CREB Regulated Transcription Coactivator 2
CVD	Cardiovascular Disease
CypD.....	Cyclophilin D
DNAm.....	DNA methylation
DSB.....	Double Stranded Breaks
ETC	Electron Transport Chain
FOXO.....	Forkhead Box Transcription Factors
GCN2	General Control Nonderepressible 2
GH.....	Growth Hormone
GH/IGF-1	Somatotropic Axis
HIF-1 α	Hypoxia-Inducible Factor 1-alpha
HIIT.....	High Intensity Interval Training
IF	Intermittent Fasting
IGF-1.....	Insulin-like Growth Factor 1
IIS.....	Insulin/Insulin-like growth factor System
LLFS	Long Life Family Study
MnSOD.....	Manganese Superoxide Dismutase

mPTP	mitochondrial Permeability Transition Pore
mTOR	mammalian Target of Rapamycin
NA	Nicotinic Acid
NAD	Nicotinamide Adenine Dinucleotide
NAM	Nicotinamide
NAMPT	Nicotinamide Phosphoribosyltransferase
NECS	New England Centenarian Study
NF- κ B	Nuclear Factor kappa-light-chain-enhancer of Activated B cells
NMN	Nicotinamide Mononucleotide
NR	Nicotinamide Riboside
P53	Tumor Protein 53
PARP	Poly (ADP-ribose) Polymerase
PGC-1 α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1
RCT	Randomized Controlled Trial
SASP	Senescence-Associated Secretory Phenotype
SC	Senescent Cells
SIRT	Sirtuin
STAC	Sirtuin-Activating Compound
T2DM	Type II Diabetes Mellitus
TCA	Citric Acid Cycle
TNF α	Tumor Necrosis Factor alpha
TRF	Time Restricted Feeding

INTRODUCTION

The healthcare provider is committed to one thing above all: maintaining the longevity and quality of their patients' lives. With advancements in medical practice and technology, humans are now living longer than ever. However, this does not necessarily mean we are living better. Symptom mediation seems to have taken precedence of treating the underlying cause. Longevity research aims to delay onset of chronic disease and disability experienced with age. The ultimate goal; to allow individuals to maintain independence and enhance their ability to continue living full lives far beyond retirement years.

Lengthening Healthspan, Not Just Lifespan

There is a critical distinction that must be made when studying longevity, and the possibility of promoting longevity in humans. At the surface, the goal of longevity research seems to be to get individuals to live for as many years as possible. This refers to the extension of 'lifespan'. However, the word lifespan is rooted in the quantity of years and fails to acknowledge the quality of life during them. The ultimate goal is to extend 'healthspan' which describes the number of years lived disease-free with optimal retention of bodily function (15). In 1980, scientist Jim Fries attempted to correlate the onset of disease to overall lifespan with his 'Compression of Morbidity Hypothesis' which argued that centenarians are able to live so long by delay of disease and disability onset into later years (104). Follow up studies have provided some support for this theory, but it is

clear that this rule does not hold true for all centenarians. While a majority of studied centenarians did delay onset of disability into their 90's, this seemed to correlate with an increased functional reserve, not necessarily avoidance of disease (104). Thus, it goes to show that while disease and aging are correlated, this is far from being the only measure of longevity. The way people reach old age is unique and heterogenous. Thus, the true challenge is how to apply longevity research findings in an individualized approach, and in a way that is practical in a clinical setting such as a primary care office.

Aging is Complex

Founding director of the New England Centenarian Study Dr. Perls described centenarians in his article titled 'Different Paths to 100' as having multiple routes to longevity including, "the survivor, the delayer, and escaper profiles' (93). This variety provides a clue to the complexity of the genetic, environmental, and behavioral factors affecting healthspan which ultimately enable someone to reach 100 or above. This conclusion is represented in the interplay amongst the numerous physiological processes currently implicated in the literature. It is not only important to have one pathway work better than the others. Each contributes to over well-being in different ways.

Current hallmarks of aging include genomic instability, loss of proteostasis, deregulated metabolism or nutrient sensing, mitochondrial dysfunction, stem cell exhaustion, and overall altered cellular communication

(71). Under each hallmark are biochemical pathways which either aid or detract from the body's ability to maintain functional reserve and delay onset of disease and disability. Amongst the most prevalent components are NAD-dependent reactions and sirtuins, mammalian target of rapamycin and the somatotrophic axis, and DNA (24, 19, 18). The overlap amongst these players is astounding and provides the true challenge in delaying aging as a certain balance must be kept. Some execute functions which promote longevity, and can be thought of as part of the 'protectome' while others' chronic stimulation is actually detrimental classifying them under the 'damageome' for the sake of this thesis. In a final addition to this complexity, certain proponents of aging are dynamic and have both 'protectome' and 'damageome' characteristics. It is crucial to demonstrate which fall under which so that interventions attempting to improve longevity do not also hurt it in the same breath. Through careful deciphering of published reviews, randomized controlled trials, and animal studies, this work attempts to provide some insight into the application of longevity research in clinical practice. Theories will be discussed individually, as well as in conjunction with others, all of which will be translated to onset of disease and alteration of bodily function. Finally, current interventions being studied will be compared for evidence of safety and for practicality in regard to patient compliance and utilization in clinics.

SPECIFIC AIMS

Specific aims on the following thesis include:

1. Explore various accepted theories of aging and their interplay; describe the environmental, proteomic, genetic and epigenetic factors involved in their mechanisms.
2. Investigate the efficacy and safety of current longevity therapeutics including pharmaceutical, lifestyle, and behavioral methods.
3. Validate longevity therapeutics and discuss the practicality of their application in medical practice.

DIAGNOSING AGE

The question, “How old are you?” refers to the amount of years passed since birth. This is considered to be one’s chronological age. While it is often correlated to functionality, maturity, and prevalence of disease, it disregards the phenotypic variation observed in older individuals. In order to better understand the changes associated with aging, a better measure is required.

Thus, researchers have shifted their focus to “biological” or “physiological” age (46). Biological age is quantified through the use of aging biomarkers defined as, “biological parameters of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age, than will chronological age”, rather than being based in time (10). Potential biomarkers for biological age that have been identified are, “epi-genetic clocks, telomere length, transcriptomic predictors, proteomic predictors, metabolomics-based predictors, and composite biomarker predictors” (53). As more interventions claiming to increase healthspan arise, it is imperative to identify which of these markers or their combination represent biological age most accurately. It should be noted that guidelines to identify these biomarkers of aging have been described by the American Federation of Aging Research as:

1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of lifespan than chronological age.
2. It must monitor a basic process that underlies the aging process, not the effects of disease.
3. It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging test.
4. It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans.

While many biomarkers have shown promise, none have met all of the above mentioned criteria. Thus, there is a continued need for longitudinal studies to improve both the efficacy and practicality of these tools (51).

Genetic Biomarkers

DNA methylation (DNAm) of CpG islands is a process by which gene expression and ultimately function is regulated. Specifically, methyl groups being present on DNA results in a chromatin configuration which prevents genes from being transcribed, inhibiting their expression (26). While it was believed that DNAm remained stable throughout life, it has been found that changes in DNAm

do occur. Ultimately, varied methylation between individuals is correlated with environmental exposures, lifestyle habits, and age.

Thus, the “epigenetic clock” was discovered. This model utilizes biomarkers of methylation, statistical regression, and adjustment for individual variation in CpGs in an attempt to accurately predict biological age. With epigenetic changes come alterations in cell differentiation, development, and homeostasis (46). Therefore, measuring DNAm differences over time may subsequently predict changes in function associated with aging.

On theme with reduced gene expression is the consideration of telomere attrition as a marker for aging. This method follows the idea that telomeres shorten during cellular replication and as a result of certain diseases causing, “limitation of stem cell function, regeneration, and organ maintenance during aging” (49). While measuring telomere length has become as simple as a blood draw, this method lacks evidence base in longitudinal mortality studies and has yet to be proven as a better predictor than chronological age of function and disease risk (76).

Proteomic Biomarkers

Instead of measuring the regulators of gene expression, other studies have attempted to quantify the genes actually expressed. Following the central dogma of DNA to RNA to protein, scientists are using transcriptomic and proteomic data and correlating them to age-related diseases and phenotypic traits. A recent study

of 4,263 individuals aged 18-95 analyzed over 2,925 plasma proteins to develop a bioinformatic approach to uncover these changes across the lifespan. It was found that throughout life, protein expression changes in waves that are associated with disease. This procedure requires only a blood draw, thus increasing its practicality as a diagnostic tool. However, many of these “proteomic signatures” also overlap with diseases independent of age raising the need for increased specificity (65). As more signatures are uncovered, this method’s ability to predict biological age will improve, and new targets to slow its progression may come to light.

Metabolomic Biomarkers

It should be considered that the genetic methods described, while useful, are a few degrees removed from the final product. The genome and epigenome give rise to the transcriptome, which provides the blueprint for the proteome, whose function produces the metabolome. Each degree of separation is accompanied by a multitude of regulatory processes which complicate attempts to model aging using the aforementioned techniques. The metabolome being closest to the biological phenotype may therefore increase accuracy and be more beneficial in efforts to reveal mechanisms of aging. The Wisconsin Registry for Alzheimer’s Prevention is a large medical record dataset of individuals with parental history of Alzheimer’s and dementia. A study done at the University of Wisconsin utilized 1,212 longitudinal plasma samples to uncover the top 100 metabolites most strongly influenced by age. Like the other methods, the

quantification of metabolites is as simple as a blood draw. However, the panel of metabolites chosen for analysis is key, as different panels have shown to draw different conclusions of one's overall well-being. In addition, metabolites are sensitive to acute changes in diet, medication, and state of well-being and are thus vulnerable to confounds. All considered, the findings of this study were consistent with previous publications, and increased confidence that the metabolome can be used to model age, and provides insight to the onset of age-related disease and dysfunction (28).

Aging Requires Multi-Faceted Diagnostics

Long term studies of centenarians such as the New England Centenarian Study (NECS) have demonstrated that those who reach age 100 or above do so in many different ways and with a variety of functional outcomes and genetic signatures (93). This was further supported by the heterogeneous physical findings in the Long Life Family Study (LLFS) of individuals over ninety and their offspring (74). While individual categories of biomarkers provide valuable insight with regard to specific functional status, it is important to recognize the complexity of aging and the need to combine methods in order to better represent an individual's health status.

Composite Biomarkers

Table 1 summarizes the findings of a comprehensive review done by Jylhava, and demonstrates the specificity of each biomarker to different aging outcomes. While the epigenetic clock and telomere length are by far the most studied, alternative methods may still hold untapped potential. As such, it is suggested that a combination of these methods will likely be utilized as standard in future estimations of biological age.

Predictor	Method	Studies, N	Age-associated outcome	References
DNAmAge	DNA methylation	100+	Mortality, frailty, cognition, physical function, self-rated health, AD, PD, cancer	Horvath (2013), Hannum et al. (2013)
Telomere length	qPCR (T/S-ratio), Southern blot (bp)	1000+	Mortality, cancer, CVD, AD, physical function, cognition	Blackburn et al. (2006)
Transcriptomic age	Gene expression	2	IL-6, urea, albumin, muscle strength, blood pressure, lipids, glucose, BMI, smoking	Holly et al. (2013), Peters et al. (2015)
Glycan age	Glycans, proteomics	1	Fibrinogen, HbA1c, BMI, triglycerides, uric acid	Kristic et al. (2014)
Protein-derived age	Proteomics	1	Low birth weight, Framingham risk score	Menni et al. (2015)
C-glyTrp	Metabolomics	1	Lung function, hip bone mineral density	Menni et al. (2013)
Metabolic age score	Metabolomics	1	Mortality, kidney function, HbA1c, hyperglycemia	Hertel et al. (2016)
Composite biomarker	10 biomarkers combined	3	Mortality, IQ, physical function	Levine (2013), Belsky et al. (2015)
Composite biomarker	19 biomarkers in a clustering approach	1	Mortality, cancer, CVD, T2D, physical function, cognition	Sebastiani et al. (2017)

AD, Alzheimer's Disease; PD, Parkinson's Disease; CVD, cardiovascular disease; T2D, type 2 diabetes; IL-6, interleukine 6; BMI, body mass index.

Table 1: Biomarkers of Aging and their Predictive Power (53).

Phenotypes of Healthy Aging

The search for biomarkers which accurately represent an individual's age and function is still ongoing. Thus, alternative methods to elucidate functionality and prevalence of diseases associated with aging are still proven to be a valuable asset. The LLFS reviewed health records of 426 families across two generations with at least one member being over the age of 90. Through this study, five ‘healthy aging phenotypes’ were developed as measures of functional reserve: “immediate and delayed recall was used to develop the healthy memory

phenotype, grip strength was used to develop the healthy strength phenotype, forced expiratory volume in 1 s was used to develop the healthy pulmonary phenotype, systolic blood pressure was used to develop the healthy blood pressure phenotype, and the following eight markers were used to develop the healthy metabolic phenotype: body mass index, waist circumference, and fasting levels of glucose, insulin, triglycerides, high-density lipoprotein cholesterol, interleukin-6, and high-sensitivity C-reactive protein” (74). Researchers of the LLFS study found that families within the cohort lacked any significant clustering of exceptional healthy aging phenotypes. Therefore, while these measures represent functional reserve well, it is not apparent that one must be exceptional in all to live longer.

The frailty index, a proportion of accumulated deficits within an individual, is another method being utilized to quantify healthy aging. While measures of functional reserve are system-specific, the frailty index takes on a global view of an individual's state of health. In a study of elderly Canadians, Mitnitski and his group found that, “deficits accumulated at 3% per year, and show a gamma distribution, typical for systems with redundant components that can be used in case of failure of a given subsystem” (80). This represents the body’s ability to compensate for deficits as we age in an attempt to maximize function. In addition, significant differences in distribution between healthy and morbid groups were also found (80). A meta-analysis of 19 studies published in 2000 or later revealed that higher frailty index was significantly associated with

higher mortality risk (60). Thus, the frailty index represents a powerful indicator of an individual's rate of aging and a predictor of all-cause mortality.

As discussed previously, aging is not the ultimate cause of mortality but the onset of disease is. The NECS observed a delay in disease and disability onset amongst those living past ninety, which provides support to the 'Compression of Morbidity' hypothesis. While disease genetic variants were still present amongst centenarians, a higher frequency of protective 'longevity enhancing variants' were found. Specific genetic variants are still being studied, and singular markers have yet to be identified as distinguishing between centenarians and normal individuals (104). Therefore, prevalence of disease and disability rather than relying solely on genetic biomarkers is advised.

Measuring the rate of change in phenotype, frailty, and disease prevalence is proven to be a good measuring of aging. In contrast to biomarkers, which require laboratory processing, these parameters are easily quantified through physical examination and patient history. In a primary care setting, this longitudinal observation is certainly possible and provides invaluable insight to the rate at which a person is aging. If found to be excessive, a physician could follow up with a composite panel of biomarkers to pinpoint which aging pathways are involved and go about combating them.

BIOMOLECULAR THEORIES OF AGING

While aging has been investigated in the past and recently sparked new interest, the majority of its elusive mechanisms remain unsolved. Traditionally, aging was excluded from theories of adaptation or genetic coding. However, modern theories fall under two umbrellas: “Programmed Theory” and “Damage and Error Theory”. Program Theory suggests that the body functions on a pre-set timeline, and, with time, bodily systems unavoidably decrease in functionality. This includes genetic, endocrine, and immunological shifts which lead to protein deficiency, metabolic imbalances, and vulnerability to disease. In contrast, the Damage and Error Theory identifies damage to bodily systems as environmental or a product of overuse (50).

Today, mechanisms of aging can be categorized under: inflammation, accumulation, genomic, metabolic, and homeostatic. These mechanisms overlay onto one another, and together mediate the progression of age (Figure 1).

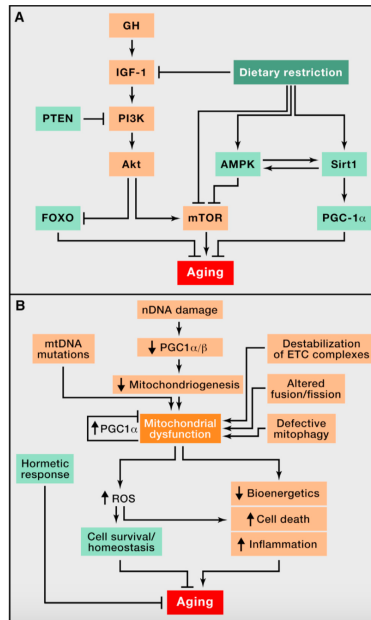


Figure 1: Interplay of Physiological Processes in Aging. (A) Overview of ‘nutrient-sensing’ pathways including sirtuins, mTOR, and the somatotropic axis. The benefits of dietary modification and caloric restriction are believed to be mediated through these proteomic players. (B) Contributors to mitochondrial dysfunction, a hallmark of aging. Altered energy metabolism due to imbalance in these processes leads to onset of chronic disease associated with age (71).

This thesis aims to not only describe them at length, but to provide a guideline to their implementation in medical practice. Pathways that enhance longevity and slow down aging mechanisms will be described as part of the “Protectome” while those which hasten aging processes will be included under the “Damageome”.

The Aging Cofactor: Nicotinamide Adenine dinucleotide

Nicotinamide adenine dinucleotide (NAD) levels dictate cell survival. This effect has been referred to as the “Mitochondrial Oasis Effect”. Specifically, reduced levels of NAD correlate with reduced survival (117). The consequences of decreased NAD have been linked to reduced function of sirtuins (SIRT1-SIRT7). These proteins have been implicated in multiple aging theories and are also believed to mediate the beneficial effects of exercise and caloric restriction described later.

NAD has noted involvement in numerous physiological processes, and over 500 enzymatic reactions. As a result, it has been implicated in energy metabolism, mitochondrial biogenesis, and DNA damage and repair (113,97). Thus, this cofactor has sparked much interest in the field of longevity and is worth examining as a proponent of the “protectome” in longevity.

The Dynamic Role of NAD

NAD plays a key role in numerous metabolic processes (Figure #). As such, any change from homeostatic NAD levels provides insight into the body’s metabolic state. Reduced levels of NAD have been associated with multiple hallmarks of aging including: mitochondrial apoptosis, reduced ATP production, impaired DNA repair, and ultimately cell death (115, 97, 34). These changes translate into numerous health issues including obesity, fatty liver disease, and metabolic syndrome (89). In contrast, increases in NAD have been shown to improve mitochondrial function under stress, prevent loss of cellular regenerative

capacity, and extend lifespan (24). Consequently, elevation of NAD levels in humans and animal models has been shown to hinder the onset of neurodegeneration, heart dysfunction, metabolic disease, and cancer (34). NAD levels are dynamic, and change in response to physiological stimuli and the circadian rhythm (24, 54). Upon change in NAD levels, comes change in the function of its protein partners and overall function of the cell. Therefore, being cognisant of level shifts, and adapting when necessary is a strategy currently being pursued in efforts to promote longevity.

NAD and Cellular Homeostasis

NAD regulates energy metabolism in response to physiologic stimuli and stressors through its role in a variety of metabolic pathways (Figure#). Glycolysis consumes NAD in the cytoplasm, the citric acid cycle (TCA) consumes NAD in the mitochondria, and finally the mitochondrial NADH is utilized by the electron transport chain (ETC) in order to generate ATP.

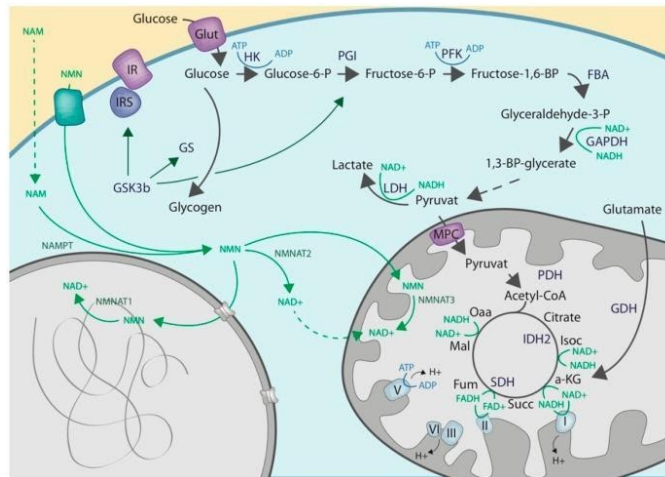


Figure 2: NAD is a critical cofactor for energy metabolism and homeostasis. Compartmental NAD/NADH levels limit ATP production. Deficiency in NAD may lead to disruption of metabolite consumption via glycolysis, TCA, and ETC. NAD levels therefore limit the capacity of the cell to produce energy and maintain

homeostatic conditions (113). Inability of a cell to maintain sufficient pools of NAD in both the cytoplasm and the mitochondria ultimately leads to cell death, regardless of the availability of glucose (24).

NAD changes cellular function through the NAD-dependent sirtuin and Poly ADP-ribose Polymerase (PARP) enzymes. Both of these enzymes, whose critical functions are discussed in the “Sirtuin” section play critical roles in metabolism, genomic stability, and overall healthspan (34). The real dilemma arises in the fact that both are ‘NAD-consuming’ proteins. Therefore, while their functions promote longevity, they ultimately deplete cytoplasmic, mitochondrial, and nuclear NAD levels (55). This emphasizes the need to boost NAD levels in older age in order to replenish these pools and maintain cellular health. Methods which boost NAD through precursors and enzymatic activation will be discussed as potential interventions to promote longevity.

Sirtuins

The protein family of sirtuins (SIRT) is central to a variety of proposed aging theories and associated degenerative diseases. Their importance stems from their sensitivity to and dependence on NAD (24). SIRT proteins are involved in ‘deacylation reactions’, and act as transcriptional regulators by targeting histones, transcription factors, and DNA repair proteins. In total, there are seven mammalian sirtuins; all localized to various cellular compartments and involved in different pathways (18). SIRT1, 6 and 7 are localized to the nucleus and act directly on histones to regulate transcription. Beyond this, SIRT 1 also acts on transcription factors and DNA repair proteins. SIRT 2 is nuclear as well, but translocates to the cytosol upon stress to activate protective metabolic enzymes. SIRT 3-5 are mitochondrial and are involved in energy metabolism, oxidative stress response, and thermogenesis (84).

Sirtuins, in particular SIRT1 and SIRT3, are nutrient sensing, and respond to NAD/NADH imbalances (88,24). Given the prominent role of NAD with regard to longevity described earlier, sirtuins are by extension prominent players as well. Their dynamic activity places them under the category of both the “damageome” and “protectome”, emphasizing the need to carefully monitor the safety of sirtuin-modulating therapeutics. While all of the sirtuins seem to play a role in cardiac and metabolic health, SIRT 1, 3 and 6 are the most well understood and targeted of the seven (55). Thus, the focus will be on their role in longevity

in the following sections. While some protein partners will be described, it is important to note that not all will be discussed for the sake of being concise.

SIRT1

SIRT 1 is currently the most targeted sirtuin for longevity therapeutics and has been associated with the benefits of caloric restriction on health (47). This may be due to the discovery that transgenic overexpression of SIRT1 has been observed to increase lifespan in yeast, *c.elegans*, flies in vivo, as well as human cells in vitro (82). As described earlier, SIRT 1 is present in the nucleus and functions by regulating gene expression directly through histone modification and modulation of transcription factor and DNA repair proteins. Due to its sensitivity to NAD/NADH levels, SIRT 1 acts as one of two ‘fuel sensor’ sirtuins (88). STRING, a database which consolidates published protein-protein interaction studies, was used to map the most prominent associations to SIRT 1, and the pathways involved (FIGURE 3, 106).

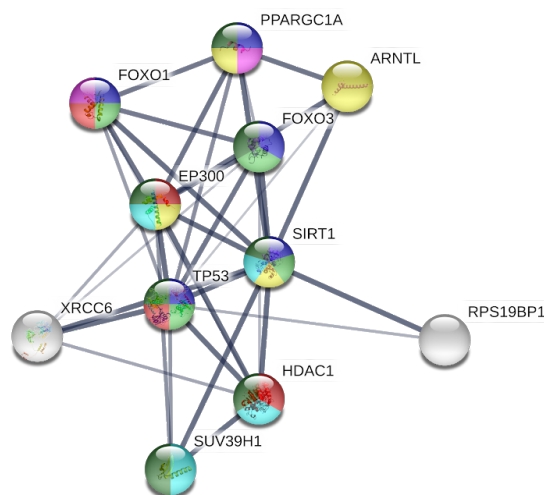


Figure 3: STRING of SIRT 1 protein-protein interactions with regard to longevity. Key protein partners are displayed as nodes. Utilizing the KEGG and Reactome databases, protein partners were filtered by relevant biochemical and regulatory pathways. Longevity (Blue), Cellular Senescence (Light Green), Cancer (Red), Insulin Signaling (Purple), Circadian Clock (Yellow), Epigenetic Regulation (Teal), and Transcriptional Regulation (Dark green). Line thickness represents confidence level of interaction based on cited literature.

Amongst the most prominent partners of SIRT1 are tumor suppressor 53 (p53), forkhead box transcription factors (FOXO), and PPAR. It has also been shown that peroxisome proliferator-activated receptor-gamma coactivator (PGC-1 α), cAMP response element-binding protein/CREB regulated transcription coactivator (CREB/CRTC2), and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) play key roles in the execution of SIRT1 function in regard to longevity (81). Stress resistance and lipid catabolism are increased via SIRT1 deacetylation of FOXOs and PPAR which are transcription factors present in the nucleus, and upregulated under oxidative stress (32). Deacetylation by SIRT1 activates 5' AMP-activated protein kinase (AMPK) and downregulates

CREB/CRTC2 to upregulate glucose and fatty acid uptake under low energy conditions in order to maintain energy homeostasis (72). PGC-1 α , a central regulator of the mitochondria, executes modulation of gene expression in response to nutrient imbalances (81). In short, SIRT1 and PGC-1 α “coordin[ate] metabolic decisions and metabolic status” (85). Given that much of the degradation associated with aging is due to excess inflammation, it is also no surprise that SIRT1 inhibits NF- κ B to limit chronic stimulation of pro-inflammatory pathways (116).

SIRT1 interaction with p53 is a bit complex. It has been proposed that SIRT1 signals successful completion of DNA repair by targeting acetylated p53, a transcription factor which arrests cell growth following DNA damage. By deacetylating p53, and in collaboration with other deacetylases, SIRT 1 abrogates apoptosis and cellular senescence and has the potential to return cells to their physiological states prior to sustaining damage (110). The complexity arises when considering the role of p53 as a tumor suppressor. Much controversy still lies in SIRT 1 has been shown to inhibit p53 in cancerous cells, therefore dampening its ability to halt tumor growth. However, studies are now being conducted in which SIRT1 antagonism is being used to treat cancer (117). This emphasizes SIRT1 characteristics of the ‘protectome’ and ‘damageome’, and thus the need to carefully monitor the safety of SIRT1 modulation as an intervention to promote longevity.

SIRT6

SIRT6, another nuclear sirtuin, has been linked to mitochondrial respiration and cellular response to oxidative stress. According to Morris, “deficiency of this other nuclear sirtuin in mice leads to low IGF-1, severe hypoglycemia, and early death... loss of SIRT6 is accompanied by genome instability. This is because during oxidative stress SIRT6 is recruited to sites of DNA double-strand breaks, where it stimulates DNA repair through both nonhomologous end-joining and homologous recombination” (81). Beyond its modification of histones directly in order to control transcription, SIRT6 plays a key role in glucose homeostasis via hypoxia- inducible factor 1-alpha (HIF-1 α) and insulin-like growth factor 1 (IGF-1), oxidative stress and DNA repair via PARP1, and management of inflammation via Tumor Necrosis Factor alpha (TNF α) and NF- κ B (120, 73, 57).

HIF-1 α is a central regulator of glucose homeostasis and acts as a metabolic switch from mitochondrial aerobic respiration to anaerobic glycolysis, a process known as the ‘Pasteur Effect’. In addition, it has been deemed a mediator of the Warburg Effect, which enables tumor cells to have increased glycolytic activity even in normal oxygen-rich conditions. (103). Numerous studies suggest that apart from hypoxia, HIF-1 α activity occurs in a growth factor dependent manner, particularly in response to IGF-1 (119). SIRT6 deficient mice die prematurely due to depleted glucose stores. HIF-1 α ’s role in the mobilization of glucose therefore became of interest as a potential effector of SIRT6 glucose

homeostasis. In Zhong's study of SIRT6 deficient mice, it was subsequently proven that SIRT6 represses HIF-1 α expression, and plays important roles in managing nutrient stress and limiting cancer growth (120).

PARP1 is involved in recognizing single and double stranded DNA breaks while moderating their repair (59). SIRT6 becomes involved when considering DNA damage due to oxidative stress, and the consequent activation of repair mechanisms via PARP1 signaling (73). In particular, it has been demonstrated that SIRT6 deficiency is associated with loss of proper base excision repair, vulnerability to DNA damage, and increased aging-associated degenerative diseases (83). Similar to SIRT1, SIRT6 also limits NF- κ B activity as well as that of the proinflammatory cytokine TNF α in response to stress, solidifying its role in limiting chronic inflammation and shedding light on its possible involvement in cancer (57). While all currently studied sirtuin-activating compounds (STAC) target SIRT1, there is some evidence to suggest that particular free fatty acids including myristic, oleic, and linoleic acids may upregulate SIRT6 activity (35). Since SIRT6 expression may be modulated, and given its involvement in these 'protectome' pathways, it is critical to keep this protein on the radar when attempting to enhance longevity.

SIRT3

A hallmark of aging is reduced mitochondrial function and energy production (71). SIRT3 is located in the mitochondria and is responsible for maintaining a state of 'mitohormesis', or mitochondrial homeostasis (88). Like

SIRT1, SIRT3 is a fuel sensor, and executes its function via NAD-dependent deacetylation of a variety of protein partners associated with cellular stress response and energy homeostasis (Figure 4). In addition, its activation is associated with the benefits seen under caloric restriction (24). SIRT3 is specifically implicated in cardiac function, and has key roles in maintaining cardiomyocyte health (3).

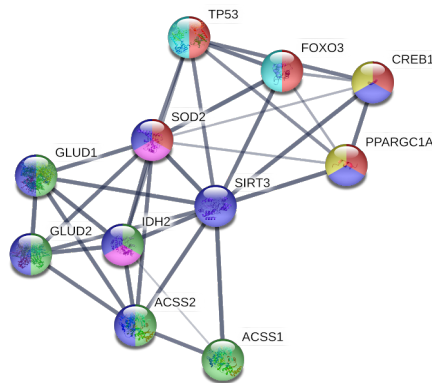


Figure 4: SIRT3 maintains mitochondrial energy homeostasis. Utilizing KEGG/Reactome pathways, a STRING diagram of protein partners was developed. SIRT3 modulates key proteins (nodes) involved in longevity (Red), energy metabolism (Green), peroxisomal function (Pink), cellular senescence (Teal), mitochondrial biogenesis (Purple), and Circadian Rhythm (Yellow). Line thickness represents confidence level of interaction supported by cited texts.

Protein partners of SIRT3 play key roles in longevity and are particularly cardioprotective, which may explain the high presence of SIRT3 in cardiomyocytes (105). These include: cyclophilinD (CypD), mitochondrial

permeability transition pore (mPTP), manganese superoxide dismutase (MnSOD), FOXO3 and DNA repair protein Ku70 (88). SIRT3 interacts with FOXO3 to protect mitochondria from oxidative stress. By clearing defective mitochondria (mitophagy), and upregulating mitochondrial biogenesis, the SIRT3-FOXO3 complex maintains functional reserve and mitochondrial density within various tissues (108). The activation of mPTP is associated with reduced mitochondrial function, increased cell death, and decline of cardiac function associated with aging (25). The SIRT3-CypD complex protects the mitochondria from cellular stressors by inhibiting the opening of the mPTP (42). Similarly, SIRT3 activates MnSOD to reduce the presence of reactive oxygen species (6). Finally, SIRT3 has been shown to promote Ku70 inhibition of Bax, a pro-apoptotic protein in cardiomyocytes. By doing so, SIRT3 prevents cardiac cell death and aids in the retention of cardiac function under stress and aging (42, 6).

SIRT3 is associated with the benefits of caloric restriction due to its regulatory role in energy homeostasis. SIRT3 coordinates substrate flow into the mitochondria and upregulates ETC function under stress. Interestingly, it has been shown through genetic knockout models that SIRT3 directly acts on Complex I of the ETC, which produces NAD from NADH. Thus, it has been speculated that SIRT3 “might act as a rheostat for the ETC ,using NAD generated from complex I activity, to, in turn, modulate overall energy homeostasis” (3). Researchers have recognized SIRT3 as a ‘protectome’ protein specifically in the context of cardiac

health, and its implication in age-related cardiac degeneration will be expanded upon.

mTOR, Insulin Signaling, and the Somatotrophic Axis

According to the “mTOR-Centric Ageing Theory”, caloric restriction and exercise result in the down-regulation of the protein kinase named mammalian target of rapamycin (mTOR) and its downstream effects. mTOR effect on cell metabolism, growth, proliferation and survival implicate it in various aging pathways as well as cancer (64). Although its role in longevity is considered controversial, data in support is widespread and highly consistent across independent studies (112). Animal studies also support that pharmaceutical inhibition of mTOR by the immunosuppressant Rapamycin (after which it is named) increases lifespan in mice (Apelo 2016). Perhaps what allows mTOR to be involved in such a variety of aging-related pathologies is its interconnection to insulin signaling and the somatotrophic axis.

The mTOR gene actually encodes two distinct complexes, mTORC1 and mTORC2. mTORC1 responds to specific amino acids (AA), hormones, as well as energetic stress and is responsible for initiating anabolic processes while inhibiting catabolic ones to promote cell growth (100). In contrast, mTORC2 regulates cellular cytoskeleton organization and is involved in insulin signaling (112). In mouse models, downregulation of mTORC1 expands lifespan, however disruption of mTORC2 seems to be detrimental (64). Therefore, the mTOR

pathway, while initially considered to only be part of the “damageome” due to mTORC1, seems to also have “protectome” characteristics when considering mTORC2 function. Therefore, there is cause for caution when considering this pathway as a target for longevity therapeutics, since complete inhibition by Rapamycin does not ubiquitously promote longevity (63).

Insulin Signaling, IGF-1 and mTOR

The insulin/Insulin-like growth factor 1 (IIS) system has been implicated in human longevity across a multitude of studies (109, 12). A common theme in pro-longevity pathways seems to be a shift from anabolic to catabolic metabolism depending on the availability of nutrients to the cell. Given IIS anabolic role, it comes as no surprise that reducing its activity may extend lifespan. Excess activation of the IIS by insulin or IGF-1 has a multitude of negative effects on health including: hypertension, atherosclerosis, and obesity. A study of 192 centenarian’s offspring has shown lower levels of plasma IGF-1 and increased sensitivity to insulin when compared to 82 non-centenarian offspring controls, providing further support for the IIS involvement in longevity (111). According to LaPlante, mTOR represents the convergence of downstream players activated by the IIS (64). Therefore, the rationale follows that by inhibiting mTOR one could reduce anabolic effects of the IIS and mimic the beneficial catabolic effects of caloric restriction on health span.

Somatotropic Axis and mTOR

Defects in the somatotrophic axis have been shown to expand lifespan in a variety of studies, particularly of dwarf model systems with growth hormone (GH) deficiency (68). GH activates the somatotrophic axis during childhood and adolescents to promote longitudinal growth, while in adulthood it functions to regulate metabolism via downstream activation of IGF-1 (16). A summary of the findings in GH-deficient mouse models show reduced circulating IGF-1 and insulin as well as increased insulin sensitivity. Overall, median longevity was increased by 43% in females and 51% in males (14).

The effect of growth hormone is important to discuss, as the use of GH supplementation is widely promoted as an anti-aging drug. The fad of age-related reduction of GH being the cause of fatigue, weakness, and reduced libido in the elderly has fueled a pharmaceutical campaign to sell synthetic growth hormone without much consideration for its detrimental effects on longevity (94). This emphasizes the importance of understanding specific mechanisms of longevity in order to protect these patients from claims of false hope and dangerous interventions.

The Human Genome and Longevity

Damage to DNA leading to protein dysfunction has been foundational to biomedical research, and aging processes are included. Aging has been found to be associated with widespread changes in the genome at both the DNA and

chromatin levels. The genome is constantly barraged throughout the lifespan from both the environment and internal processes. This results in genetic damage in the form of, “point mutations, trans locations, chromosomal gains and losses, telomeres shortening, and gene disruption caused by the integration of viruses and transposons” (71). Deficits associated with aging come about when DNA damage accumulates past a cell’s capacity to repair it, resulting in disruption of proper gene expression, cellular senescence, and consequently disease (58). Although these theories provide some explanation to age-associated health decline, it is important to recognize that gene therapy in humans is still a new and developing field. Therefore, while genetic manipulation may be an option in the distant future, it is not a verifiably safe intervention to promote longevity in the present. For now, preventative measures can be taken to minimize the amount of DNA damage that is accumulated in hopes of promoting longer healthspans.

Telomere Shortening

A pillar in aging, “Telomere Shortening” argues that aging is a consequence of reduced telomere length with successive cell cycles. Particularly in the mitochondria, evidence demonstrates increasing instances of mitochondrial heteroplasmy and resultant dysfunction with old age and shortened telomere length (121). According to a study utilizing telomerase knockout mice, excessive telomere shortening lead to downstream SIRT repression by p53, and modeled premature aging. Furthermore, upregulation of SIRT activity either directly or through NAD boosting proved to stabilize telomeres (5). The relationship

between healthspan-protective SIRT6 and telomere stability hints at the involvement of telomeric attrition in age-related decline and mitochondrial dysfunction.

Epigenetic Change

The “Relocalization of Chromatin Modifiers Hypothesis” proposes that epigenetic changes in DNA in response to double stranded breaks (DSBs) accelerates aging, and has been proven in an Inducible Epigenetic Change mouse model (55, 58). These epigenetic changes, thought to be caused by chromatin remodeling in response to DSBs, are believed to underlie cellular senescence in age-related functional decline (58). Causes of these mutations include air pollutants, UV exposure, drugs, diets and more (9).

The ‘epigenetic clock’ was described earlier as a marker of aging based on methylation of CpG islands. This is not only limited to nuclear DNA, but mitochondrial DNA as well. While the association of epigenetic change with aging and a variety of disorders has been demonstrated, the exact mechanism of degeneration is still yet to be elucidated (9). However, as more is uncovered, manipulation of DNA methylation may prove to be a probable intervention to promote longevity.

INTERCONNECTION OF AGING THEORIES IN AGE-RELATED DISEASES

So far, we have discussed the bimolecular mechanisms believed to be involved in both the delay of aging and its acceleration. These included the regulatory role of NAD categorized under the ‘protectome’, the highly dynamic sirtuins categorized under both the ‘protectome’ and ‘damageome’, the mTOR/IIS signaling network categorized under the ‘damageome’, and the accumulation of genomic instability. The categorization of each translates into their implication in a variety of common age-related diseases. As one ages, the ‘protectome’ pathways are disrupted while the ‘damageome’ pathways become aberrantly activated. To demonstrate this, the basis of five diseases in which age is considered a major risk factor will be described including: chronic inflammation, metabolic disorders, cardiovascular disease, neurodegeneration, and finally cancer (86, 47, 81).

Cellular Senescence, Chronic Inflammation and Neurodegeneration

Chronic inflammation is characteristic of a multitude of common aging and autoimmune diseases. Cellular senescence, or a state of cell arrest, is believed to be a major factor implicated in this phenotype (48). Apart from inhibiting cellular proliferation, this state is also associated with a pro-inflammatory senescence-associated secretory phenotype (SASP). Senescence occurs in response to stress, but also due to genomic instability, loss of proteostasis, and

loss of mitohormesis; all of which were associated with the dysfunction of the molecular theories of aging described earlier (98). In particular, SASP is dependent upon known protein partners of sirtuins and mTOR including NF- κ B, p53, AMPK, and IGF-1. Furthermore, SASP is associated with elevated levels of chemokines, cytokines, and growth factors which are deemed as markers of ‘inflammaging’, or age-related chronic inflammation (61). To add insult to injury, as senescent cells (SC) accumulate, neighboring cells become affected by their proinflammatory factors, hence the term ‘zombie cells’ (102). Increased quantities of SC cells have been associated with chronic inflammation and neurodegenerative disorders including Alzheimer’s and Parkinson’s disease (61, 38, 22).

SIRT6 moderate both adaptive and innate immunity in response to T-cells (81). In an interview with longevity physiologist Peter Attia, Dr. Sinclair explained that as genomic damage is accumulated throughout life, sirtuins are constantly being relocalized in an attempt to repair it. However, there is a point when sirtuins are ‘spread too thin’ and can no longer compensate (1). Specifically, SIRT1 and SIRT6 inhibit NF- κ B transcription to limit the body’s inflammatory response (116, 57). Therefore, impaired sirtuin activity allows acute inflammation to progress into the chronic pathologic inflammation observed in disorders such as osteoarthritis.

SCs can also be triggered by oxidative stress observed in dysregulated mTOR signaling. mTOR inhibition by rapamycin has been shown to reduce

interleukin 1 receptor mediated SASP via transcriptional suppression of NF- κ B (66). Furthermore, mTOR activation due to defective growth factor and AA signaling leads to loss of mitohormesis and the production of ROS-induced genotoxic stress seen in SCs (90). There is also evidence that sirtuins, particularly SIRT1, downregulates mTOR activity in response to stress (45). Considering this interplay between the highly implicated SIRT and mTOR, and given mTOR's role as a key component of mitochondrial biogenesis and IGF-1 signaling, it is plausible that dysregulation of mTOR may underlie chronic inflammation seen in aging.

Metabolic Disorders and Cardiovascular Disease

Metabolic decline is another proponent of aging currently being studied. The founder of the Institute for Aging Research, Dr. Barzilai, argues that "aging is arguably the most universal contributor to the etiologies of metabolic decline including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and stroke" (14). Studies suggest that throughout our lifespan, metabolism shifts into a more anabolic state. This is characterized by increased adiposity and a state of insulin resistance coupled with excessive glucose production known as metabolic syndrome. In a meta-analysis of 20 cohort studies including over fifty thousand patients, researchers found metabolic syndrome to be associated with higher risk of all cause mortality in the elderly (52). Similarly to chronic inflammation, this

impairment of metabolism implicates multiple aging theories. Thus, the complexity of why we age is once again emphasized.

The altered metabolism associated with aging involves the interplay between sirtuins, mTOR, and the somatotrophic axis (14). As mentioned earlier, sirtuins inhibit PPAR to limit its adipogenic effects in response to changes in nutrients. Given that PPAR activity helps determine age-related insulin resistance, SIRT1 appears to be the link between diet, body fat, and age-related diseases (32). In addition, SIRT1 deficient mice lack the ability to regulate mTOR signaling. Excessive mTOR activity, and the crosstalk with IIS signaling results in insulin resistance and a loss of glucose homeostasis which are both characteristic of the aforementioned diseases (81).

Also believed to have a role in aging is the somatotrophic axis (GH/IGF-1). There is much debate on whether the decrease in GH/IGF-1 or its increase is best for longevity. Dwarf mouse models which lack the GH receptor show significant extension of life, and mutations in the IGF-1 receptor gene are more common in centenarians than the young (68). Paradoxically, low circulating IGF-1 has also been associated with increased risk of CVD and other metabolic-mediated diseases. This has led researchers such as Barzilai to conclude that attenuation of the GH/IGF-1 axis must be optimized, not necessarily abolished, as it contains both 'protectome' and 'damageome' qualities (14).

Cancer

Aging is also considered a risk factor for cancer, and involves altered signaling of the three sirtuins discussed (SIRT1/3/6) as well as the mTOR pathway (81, 14). However, their effect on cancer is not unidirectional, and attenuation of their function should therefore be approached cautiously. Each protein's role in the cancer pathology can be uncovered by revisiting key protein partners and their downstream effects. This includes SIRT1 interplay with the tumor suppressor p53, SIRT6 response to DNA double stranded breaks, SIRT3 regulation of HIF-1 α , and mTOR upregulation of mitochondrial respiration (117, 73, 101,90).

SIRT1 is a known inhibitor of the tumor suppressor p53, and is upregulated in a variety of cancers (47). However, downregulation of SIRT1 is also associated with excessive DNA damage and loss of genomic stability which leaves cells vulnerable to mutations and tumorigenesis (117). Therefore, both SIRT1 activators and inhibitors are being considered as potential cancer therapeutics, yet it seems as though a dynamic regulator of SIRT1 which would allow it to function at an optimal level is needed. SIRT6 is a central player in regulating DNA repair of double stranded breaks under stress, primarily via activation of PARP1 (32, 73). In contrast to SIRT1, SIRT6 acts solely as a tumor suppressor by preventing the accumulation of DNA damage caused particularly by oxidative stress (73). Since genomic instability is a basis for tumor production,

it comes as no surprise that SIRT6 has now become a target of cancer therapeutics.

Cancer cells mandate a high amount of energy, and require an upregulation of anaerobic metabolism to survive in their overcrowded, hypoxic environment (4). Involved in this metabolic shift is HIF-1 α which is regulated by SIRT3 and mTOR signaling. Normally, SIRT3 inhibits HIF-1 α stimulation of glycolysis in response to oxidative stress. However, SIRT3 deficiency observed in human breast cancer allows for unregulated HIF-1 α activation even under normoxic conditions. This results in upregulation of glycolysis (Warburg Effect) as part of a pseudo-hypoxic response which nurtures tumor cell growth and proliferation (101). Similarly, mTOR is also upregulated in response to oxidative damage. It's activity, particularly mTORC1, stimulates mitochondrial respiration and increases ATP production to fulfill the energy requirements of highly proliferative cancer cells (90). Furthermore, it is also associated with HIF-1 α activation like SIRT3 (66). As such, modulation of mTORC1 activity is also considered to be a potential anti-cancer therapeutic. However, not much is known about the role of mTORC2, which further complicates the targeting of this complex which will be discussed in the next section.

INTERVENTIONS TO PROMOTE LONGEVITY

Humans have been looking for the “fountain of youth” for centuries. According to University of Delaware historian Carol Haber, the search began between the 15th and 16th centuries with the intention of preserving the elderly’s ability to pass on precious knowledge. Then, between the 19th and 20th centuries, a shift began to take place (41). The goal then became to not age at all.

Modern longevity research continues to toe the line between improving quality of life into older age and ostracising the aged. As “anti-aging” hype continues to snowball, it becomes critical for providers to be aware of current research on aging interventions in order to protect a vulnerable geriatric patient population. Things such as unregulated supplements, off-label use of pharmacologic agents, and hormone replacement being marketed directly to consumers without proper safety standards are becoming more and more prevalent. However, while some interventions have yet to be proven, others have demonstrated promise in the efforts to slow down aging and preserve function.

This section aims to identify current interventions whose efficacy and safety are largely supported by translational research and clinical findings. These interventions can be categorized into the following categories: lifestyle habits, pharmacologic agents, and orthomolecular medicine. It is important to note that in the case of longevity, it is difficult to conduct comprehensive longitudinal studies primarily due to their length and subsequent cost. In addition, medical practitioners must be aware of greed driven predation of the elderly patient

population. Dr. Perls, the founding director of the NECS, wrote in an Aging Health article of the four major events leaving older patients vulnerable to potentially dangerous therapeutics: a growing population of baby boomers searching for a fountain of youth, television shows advertising unproven therapies with a false backing by celebrities, loopholes in the Dietary Supplement and Education Act allowing politicians to market unregulated products directly to consumers, and finally underfunding of governmental enforcement agencies (95). Therefore, cautionary measures will also be discussed.

Targeting the ‘Dynamic Duo’: NAD and Sirtuins

As described earlier, NAD and the NAD-dependent sirtuins are central to a multitude of physiological functions and aging processes. Thus, many groups are now searching for ways to boost their activity. However, sirtuins are involved in a multitude of longevity pathways, some under the umbrella of the ‘protectome’ while others fall under that of the ‘damageome’. A leader in NAD boosting and sirtuin activating therapies, Dr. Sinclair, describes sirtuin activity as a ‘double-edged sword’, emphasizing the need for rigorous testing for safety and dosage (18). Today, there are several NAD precursors and STACs being tested in clinical trials, the most promising of which will be described.

Boosting NAD: Niacin, NR, and NMN

The methods by which we can raise cellular NAD levels are rooted in the synthesis, salvage and metabolism of NAD itself. NAD is produced in the

cytoplasm, nucleus and mitochondria (55). NAD is synthesized de novo in the cytoplasm from dietary tryptophan in a process known as the kynurenine pathway. NAD salvage occurs in the cytoplasm as well as the mitochondria and nucleus from recycled precursors: nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), nicotinamide (NAM), and nicotinic acid (NA)

(97). The possibility to upregulate mitochondrial and nuclear NAD production is based in the fact that these precursors may be transported across plasma membranes for utilization (Figure 5).

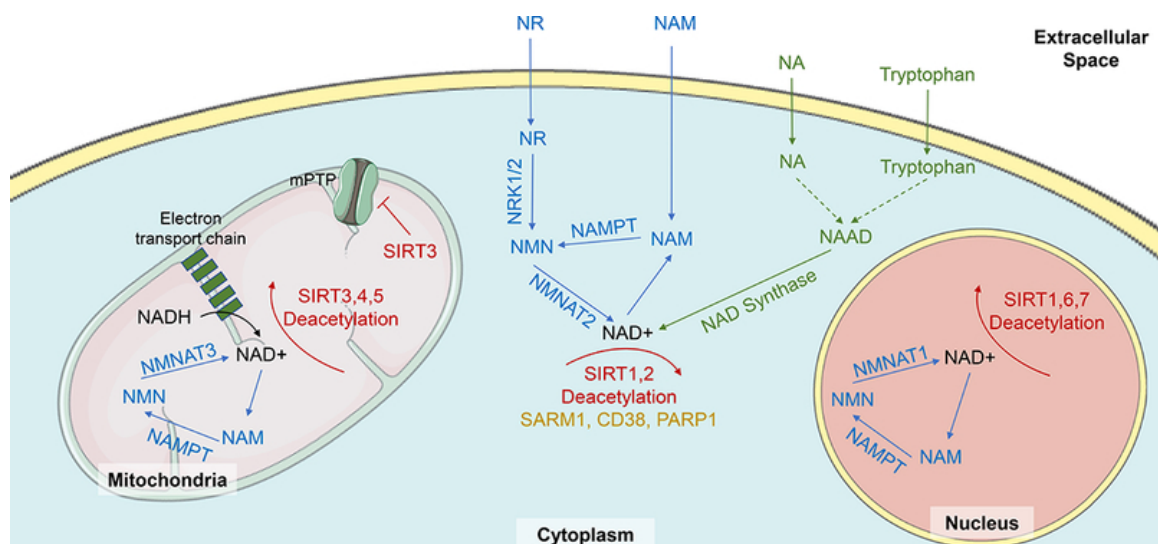


Figure 5: NAD production in different cellular compartments. NAD is produced de novo in the cytoplasm or via salvage pathways in the cytoplasm, nucleus, and mitochondria. Current interventions to promote longevity include addition of precursors and subsequent upregulation of salvage pathways (Blue), direct activation of nicotinamide phosphoribosyltransferase (NAMPT), and inhibition of NAD-consuming enzymes SARM1, CD38, and PARP1 (55).

Niacin, used to describe nicotinic acid (NA), is a form of vitamin B3

initially used to treat arteriosclerotic cardiovascular disease due to its ability to

increase the amount of high density lipoprotein (37). Niacin being a precursor to NAD made it a molecule of interest in the efforts to increase longevity. However, while it was proven to raise NAD levels in rodents, there is little data supporting its ability to do so in humans (97). Noted side effects of niacin include, “impaired glucose tolerance, rise in uric acid, and, more rarely, rises in liver enzymes and myopathy” (56). Furthermore, a systematic review of thirteen RCTs of 35,206 individuals noted no significant difference of all cause mortality between niacin and control arms as treatment for reducing cardiovascular events (37). Therefore, niacin does not represent a good candidate for the promotion of longevity.

NR, another proponent of the vitamin B3 family, proves to be a more promising precursor to boost NAD. Niagen® is an NR which has been studied in several clinical trials to date, and is being brought to market by TRU NIAGEN. A 2 x 6 week randomized controlled trial (RCT) of Niagen® in healthy middle aged adults demonstrated its ability to stimulate NAD levels in the blood without adverse effects (75). According to TRU NIAGEN, no known drug interactions are expected. This is critical when considering the ever prevalent polypharmacy present in the elderly patient population (43).

NMN is another NAD precursor being used to upregulate NAD production in cells. NMN is synthesized during the rate-limiting step of NAD synthesis under the function of NAMPT. It is also made from NR via NR kinase activity, shedding light on Niagen’s ability to bypass the rate-limiting NAMPT (17). According to a review by Yoshino, NMN improves insulin sensitivity,

mitochondrial function, neuro cognitive function and endothelial function in mice (118). Moreover, neuroprotective compounds P7C3 which activate NAMPT have also been found to upregulate NAD production via this salvage pathway which provides supporting evidence to NMN's ability to limit neuronal degeneration (18). According to the US National Library of Medicine, there is only one ongoing clinical trial of NMN effect on cardiometabolic function, therefore physicians should hesitate to recommend the use of this supplement which is commercially available. As more studies will be conducted, it is worthwhile to keep NMN on the radar of potential longevity interventions.

Sirtuin-Activating Compounds

Sirtuins have been shown to mediate the health benefits of caloric restriction and are implicated in age-related diseases in a multitude of studies (47, 82, 15). STACs are pharmacological activators of sirtuins which, like NAD precursors, seem to delay aging and prevent the onset of age-related diseases and disability (47). The first STAC to be discovered and studied was resveratrol, a polyphenol found in red wine and grapes. Due to the high cost on long-term clinical studies, clinical trials of this STAC have been limited to disease-specific and short term studies. Through these studies, resveratrol has been described to have antioxidant, anti-inflammatory, and anti-neurodegenerative properties which are protective against cardiovascular, metabolic, and cognitive decline (15). However, resveratrol also presents a few risks which make it difficult to justify its widespread use in clinics. These problems include low absorption and

bioavailability, lack of specificity to SIRT1, and disruption of normal drug metabolism due to cytochrome P450 inhibition which poses an even greater risk to the elderly who commonly take multiple medications at once (39, 30). Early synthetic activators such as SRT1720 also had low target specificity and thus have not made it into practice (18). The search for a specific and bioavailable compound is still ongoing.

Currently, a number of synthetic pharmacological activators of SIRT1 are being tested in clinical trials. GlaxoSmithKline, a british multinational pharmaceutical company, is currently studying three synthetic STACs including SRT2104, SRT2379, and SRT3025 in hopes of gaining approval for clinical use (27). According to a review by Dr.Dai, only SRT2104 was found to have potential longevity promoting effects while SRT2379 and SRT3025 studies have subsequently been discontinued (27). RCTs of SRT2104 have shown some promise. In psoriasis, an inflammatory disease, SRT2014 was demonstrated to reduce inflammation by modulating TNF α activity (62). A study of T2DM patients showed that while SRT2104 may not have an effect on glucose or insulin signaling, it does improve lipid profiles (11). Finally, a pilot study in elderly volunteers showed that SRT2104 is tolerable and mimics the benefits associated with increased SIRT1 activity (67). However, all of these studies pointed out that while there are noted benefits, SRT2104, like the other STACs mentioned, is not able to overcome bioavailability and specificity issues. Thus, there is still a need

to find a compound which is absorbed well and accurately targets SIRT1 without off-target effects.

Senolytics: Fighting ‘Zombie Cells’

Cellular senescence and the associated SASP phenotype has been implicated in various age-related diseases. Therefore, current studies aim to selectively eliminate these cells primarily by targeting their pro-survival mechanisms which allow them to avoid apoptosis in the first place (44). Agents which aim to accomplish this are categorized as Senolytics. “Hit and Run” treatment with senolytics such as the Dasatinib/Quercetin cocktails have been demonstrated to reduce p16^{INK4A} and p21^{CIP1} expressing cells (SC cells) in both idiopathic pulmonary fibrosis and diabetic kidney disease. Noted Improvements included increased tolerance to exercise and a reduction in inflammatory markers (44, 47). It is important to note that both of these studies were modest in size with 9 patients being included in the diabetic kidney disease trial and 14 in the IPF trial. In addition, neither study observed significant improvements in frailty index or self-reported states of well-being. Therefore, there is still a need to find senolytics which can promote longevity not only in those suffering from chronic disease, but in healthy individuals as well.

Other Pharmaceuticals

Rapamycin

The mTOR pathway has numerous implications in the acceleration of aging by promoting cell proliferation, growth, and inflammation. Interestingly, mTOR was named after the immunosuppressant, Rapamycin, which used to be considered an antifungal agent until its inhibition of T-cell proliferation was discovered and connected to the mTORC complexes (7). While both complexes fall under the same gene, their varied response to Rapamycin is critical for understanding the risk and benefit of utilizing this drug as a longevity intervention. Specifically, mTORC1 is inhibited by acute dosing of Rapamycin and has been shown to extend lifespan in yeast, flies, and mice (7). Usually, mTORC1 allows cancer cells to keep up with high energy demands under hypoxic conditions, however Rapamycin treatment has been shown to prevent this survival mechanism making it a potential anti-cancer therapy. In addition, inhibition of mTORC1 has been shown to be protective against Alzheimer's, Parkinson's, and Huntington's in animal models (66). However, chronic administration has been shown to inhibit mTORC2 function and results in glucose intolerance and insulin resistance (63, 19).

Rapamycin's effect is variable across different organisms and lacks efficacy. To solve this, combination therapies are now being constructed to better target particular pathways. These molecules, called Rapalogs, target specific receptors to increase Rapamycin inhibition including: IGF-1R, receptor tyrosine kinases, and growth factor inhibitors (66). Currently, the use of Rapamycin in

healthy patients has yet to be proven safe, and more work must be done before its utilization in clinics.

Metformin

Metformin is a pharmaceutical agent which lowers blood glucose, and is currently being used robustly to treat T2DM (71). When it was observed that diabetics taking metformin had lower mortality than non-diabetic not taking metformin, it came into focus as a potential pro-longevity therapeutic. A meta-analysis by Dr. Campbell concluded that Metformin users had a reduction in instances of CVD and cancer (23). A study of clinical trials also noted reduction in these diseases, as well as a reduction in neurodegeneration, inflammation, and frailty (96). The ‘geroprotective’ properties of this drug may be attributed to its ‘fasting mimetic’ properties such as activation of AMPK to reduce excessive glucose production, inhibition of methionine metabolism which is linked to extended lifespan, and finally improved response to insulin to prevent the deleterious effects of T2DM (71). Given the potential benefits of metformin beyond just treating diabetes, Barzilai and collaborators from AFAR have set out to prove its safety and pharmacodynamic efficacy through TAME; the Targeting Aging with Metformin clinical trial (107). If the trial is successful, not only will metformin become an approved drug to delay aging, it will change its definition all together and classify it as an indication, which will hold vast weight when considering public health and insurance stipulations. Overall, metformin seems to

be a promising pharmaceutical which may be utilized in practice beyond just diabetes within the next 10 years.

Dietary Modification

Organisms, under the 24-hour dark/light cycle, have evolved to develop a 24 hour circadian rhythm. Amongst many other things, it is an internal signal to acquire food when it is available, and utilize stored energy when it is not without compromising vitality. Thus the question arose; what mechanisms enable the retention of function without additional energy intake? Through studies of religious groups practicing periodic fasts, animal models, and lower eukaryotes it was discovered that fasting not only triggers compensatory mechanisms which retain normal function, but promotes longevity (77).

To understand the benefits of dietary restriction and fasting, one must first understand the mechanisms and observed benefits of 'fasting physiology' as they relate to pillars of aging. According to a recent review published in the New England Journal of Medicine, "intermittent fasting elicits evolutionarily conserved, adaptive cellular responses that are integrated between and within organs in a manner that improves glucose regulation, increases stress resistance, and suppresses inflammation" (29). These benefits occur in response to changes in the nutrients described earlier, NAD, ATP and Acetyl CoA, all of which are converted to NADH, AMP, and CoA in the fasted state. Upon this shift in energy state, transcription factors implemented in a multitude of longevity pathways are

activated. This includes the FOXOs, PGC-1 α , AMPK, and SIRT6. In addition to the upregulation of these “protectome” pathways, “damageome” pathways such as Insulin-IGF-1 and mTOR are downregulated due to a lack of circulating AA, glucose and insulin. This shift from anabolism to catabolism limits the use of resources for growth and, “favors maintenance and repair systems, enhancing stress resistance, recycling damaged molecules, stimulating mitochondrial biogenesis, and promoting cell survival” (29). Thus, researchers have conducted a multitude of studies and clinical trials in hopes of uncovering what regimens best promote longevity. In the following sections a select number of regimens currently believed to be most beneficial to longevity will be described. These regimens vary in not only amount of calories consumed, but time of feeding and actual food content.

Intermittent Fasting: When to Eat and How Much

Intermittent fasting (IF) describes, “eating patterns in which individuals go extended time periods (e.g. 16-48hr) with little or no energy intake, with intervening periods of normal food intake, on a recurring basis” (77). According to a review on the metabolic effects of IF, there are three methods commonly being utilized to impact health. These include alternate day fasting, modified fasting, and time restricted feeding (TRF) (92). Alternate day fasting involves days without any energy intake coupled with days of free eating. The modified regimen is similar except there is a 20-25% energy intake on fasting days; this is

the basis of the popular 5:2 diet which restricts energy intake for 2 days and allows free eating the other 5. Finally, TRF pertains to free eating within specific time frames broken up by longer periods of fasting; this is commonly practiced in a 16 hour fast to 8 hour eating frame structure (92). Each regimen is associated with a variety of benefits and levels of practicality which should be considered on an individualized basis.

Alternate day fasting is an example of a method which presents metabolic benefits, but is simply not practical. In both rodents and in a limited amount of human clinical studies it was demonstrated that this regimen reduces triglyceride production post-meals, elevates HDL while lowering LDL, and shows improvement in inflammatory biomarkers (92). However, Dr. Heilbronn who studies fasting regimens in both obese and non-obese adults points out that many complain of increased hunger without relief over time while undergoing this regimen (91). Therefore, this method is not very popular and lacks consistent patient compliance.

The modified regimen seems to have come about as a solution to the hunger experienced in the alternate day fasting routine. Evidence for this diet's effect on metabolism and longevity is, however, limited. A 19 subject randomized study found that under the 5:2 regimen, individuals undergo a metabolic switch to catabolism represented by a reduction in fasting glucose and body fat, as well as an increase in ketone bodies and lean mass relative to whole body weight (20, 70). Furthermore, low reports of adverse effects such as hunger indicate that this

regimen not only produces the benefits of caloric restriction, but is also less difficult to comply with.

TRF is seemingly the most studied method of intermittent fasting. TRF was founded within the concept of circadian rhythms, and exploits the body's transcriptional and translational feedback loops rather than nutrient composition to modulate metabolism (70). The circadian clock activates catabolic and anabolic pathways depending on fasted and fed state prevalent in ancient species who ate during the day and fasted throughout the night. However, in today's society where food is always readily available, the body remains at a steady fed state which negates the potential benefits of fasting physiology (70). TRF caloric restriction optimizes the fast/feed cycle already conserved in our genome, and allows individuals to optimize its benefit. Overall, TRF downregulates mTOR/IGF-1 signaling while upregulating SIRT activity which are all associated with improved longevity. Individuals undergoing this regimen show minimization of anabolic processes, increased DNA repair, enhanced stress resistance, and improved autophagy and recycling of cellular components (29). Compliance with TRF does not seem to be an issue, and its benefits are similar to that of the 5:2 diet. Given that neither have been proven harmful, there seems to be no strong preference for one or the other amongst researchers. Thus, both represent valid and useful tools to reduce metabolic dysregulation, chronic inflammation, and cardiovascular disease associated with aging (70).

Dietary Restriction: What to Eat

In contrast to caloric restriction and intermittent fasting, another school of thought follows components of dietary restriction. This involves limiting the intake of protein and specific AA in order to target nutrient signaling pathways involved in longevity. Specifically, limiting the intake of serine, threonine, methionine, and protein has been shown to extend lifespan in yeast and animal models (79, 99, 78). Implicated in the benefits of dietary restriction is the AA regulator general control noderepressible 2 (GCN2) and the mTOR/IIS axis (79).

Serine and threonine came to light as pro-aging when it was discovered that cells become overly sensitized to oxidative stress with these AA in medium. Serine and threonine promote mTOR activation and ultimately inhibit stress resistance protein kinase Rim15 (20). In addition, AA scarcity activates GCN2 which has been shown to promote longevity as well. Methionine restriction is also involved in this pathway, in particular it reduces IGF-1 signaling, described earlier to promote longevity as well. Common diets which naturally limit intake of these AA include vegan diets, plant proteins, and moderate intake of beans, legumes, and soy (78). Therefore, not only controlling when you eat, but what you eat could optimize the function of pro-longevity functions, and allow the body to reap the fruits of ancient and conserved evolutionary mechanisms for survival.

Targeted Exercise

It is a common belief that regular exercise is beneficial to overall health, both physical and mental. Applied physiologist and leader in longevity-based medicine Peter Attia has even challenged his followers to take on the ‘Centenarian Olympics’; a regimen of targeted workouts designed to conserve functionality into later years. Kettlebell swings to mimic lifting up a child, squats to conserve the ability to sit and stand independently, and cardio exercise for cardiac health are just a few examples (33). However, the true benefit of these exercises is not rooted in muscle memory or motor control, but the regimen’s effect on energy metabolism, stress management, and overall muscle mass which translates improved stamina, reduction in DNA damage, and resistance to frailty (36). RCTs have shown that a combination of aerobic exercise and resistance training improve diet-induced weight loss, VO2max, cancer-suppression, inflammatory suppression, and scores in modified physical performance test in frail older adults (2,87).

Particular exercise regimens which are considered to promote longevity include high intensity interval training (HIIT) and progressive resistance training. HIIT involves exercise-induced increase in heart rate to more than 90% interrupted by periods of rest. A systematic review of over 2000 studies noted improvements in CVD biomarkers in children and adolescents, and concluded that HIIT is an effective time-effective intervention to promote health (31). Progressive resistance training involves 10 weighted exercises for 10-15

repetitions at moderate intensity (2). Benefits include muscular synthesis, improved physical function, and overall amelioration of frailty (40). By modifying exercise depending on current ability, and encouraging consistency, physicians can empower their patients to take their health into their own hands in a safe way.

CONCLUSION

There is no ‘one-size-fits-all’ method to promote longevity in individuals. If the age of lightning fast-genomics and big data have shown us anything is that we are each infinitely unique in our own right. Personalized medicine is on a constant rise to being the status quo of healthcare, acknowledging that our uniqueness demands unique care. That being said, there are some things you can do today to try and secure a healthier and more independent future.

It is now clear that aging is not something to fear or ostracize. With older age come wisdom, experience, and life lessons critical to future generations. Therefore, there is much to gain by empowering the elderly to continue to contribute to society and maintain independence. There is no rule that says aging must be accompanied by suffering. Studies of numerous aging theories have shown that the way we age is something that can be modulated, delayed, and therefore improved. Key proteins players such as the sirtuins and mTOR are being described and targeted in new studies all the time. Genetic manipulation is another exciting and new field which may yet prove to contribute to longevity therapeutics as well. Perhaps chronic diseases such as CVD and dementia may become less prevalent in the future as the shift from symptom-based care to preventative medicine continues to take hold in hospitals and medical schools alike.

The easiest things to implement into our routine in order to promote longevity are those things which we can personally control; the way we diet and

exercise. The evidence in support of intermittent fasting with little negative effects beyond feelings of hunger is only growing. Just by restricting when you eat, and how much you eat in a day can jump start your journey to becoming an active grandparent. Simple cardiovascular exercise for twenty minutes three times a week is low-cost in the sense of time and money but unlocks incredible benefits for cardiac and metabolic health. Overall, the idea of ‘staying active’ seems to be key. The body follows a “use-it-or-lose-it” mantra, and so stimulating your metabolism by caloric and dietary restriction, undertaking consistent cardio and resistance exercise, and challenging one’s cognition by continuing to pursue new accolades seem to all contribute to improved state of well-being in later years. Pharmaceuticals and supplements, for the time being, should be something to be wary of. Longitudinal studies for efficacy and safety are still underway for compounds such as STACs, metformin, and Rapamycin and although time is always of the essence with aging, jumping the gun on these interventions before they are proven is a risk.

I encourage you, the reader, not only to challenge yourself to age better but those around you. In life, it may seem that past a certain point whatever damage has been done is not worth attempting to resolve, and whatever bad habits we formed are far beyond repair. I urge you to look past this stark mentality, and believe in the resiliency of not only the body but the human spirit. Start with small steps. Stop eating between 10pm and 10am by skipping breakfast, push that 10am mark 1 hour a day until you are only eating after 2pm and just like that you begin

to reap the benefit of fasting. Walk two miles every other day until eventually you begin to run through them. Surround yourself with those who uphold a positive mental attitude and inspire you to better yourself. Just like that, you begin to take control of not only your current health, but what your future may look like. For all you know, those two miles may one day translate into 26.2 as you cross the finish line to a marathon at the age of 100.

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CURRICULUM VITAE

